Effect of coronary atherosclerosis and myocardial ischemia on plasma levels of high-sensitivity Troponin T and NT-proBNP in patients with stable angina

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Abstract: OBJECTIVE: Circulating levels of high-sensitivity cardiac troponin T (hs-cTnT) and N terminal probrain natriuretic peptide (NT-proBNP) are predictors of prognosis in patients with coronary artery disease (CAD). We aimed at evaluating the effect of coronary atherosclerosis and myocardial ischemia on cardiac release of hs-cTnT and NT-proBNP in patients with suspected CAD. APPROACH AND RESULTS: Hs-cTnT and NT-proBNP were measured in 378 patients (60.1±0.5 years, 229 males) with stable angina and unknown CAD enrolled in the Evaluation of Integrated Cardiac Imaging (EVINCI) study. All patients underwent stress imaging to detect myocardial ischemia and coronary computed tomographic angiography to assess the presence and characteristics of CAD. An individual computed tomographic angiography score was calculated combining extent, severity, composition, and location of plaques. In the whole population, the median (25-75 percentiles) value of plasma hs-cTnT was 6.17 (4.2-9.1) ng/L and of NT-proBNP was 61.66 (31.2-132.6) ng/L. In a multivariate model, computed tomographic angiography score was an independent predictor of the plasma hs-cTnT (coefficient 0.06, SE 0.02; P=0.0089), whereas ischemia was a predictor of NT-proBNP (coefficient 0.38, SE 0.12; P=0.0015). Hs-cTnT concentrations were significantly increased in patients with CAD with or without myocardial ischemia (P<0.005), whereas only patients with CAD and ischemia showed significantly higher levels of NT-proBNP (P<0.001). CONCLUSIONS: In patients with stable angina, the presence and extent of coronary atherosclerosis is related with circulating levels of hs-cTnT, also in the absence of ischemia, suggesting an ischemia-independent mechanism of hs-cTnT release. Obstructive CAD causing myocardial ischemia is associated with increased levels of NT-proBNP.

DOI: https://doi.org/10.1161/ATVBAHA.115.306818

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DOI: https://doi.org/10.1161/ATVBAHA.115.306818

Effect of Coronary Atherosclerosis and Myocardial Ischemia on Plasma Levels of High-Sensitivity Troponin T and NT-proBNP in Patients With Stable Angina

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Objective—Circulating levels of high-sensitivity cardiac troponin T (hs-cTnT) and N terminal pro brain natriuretic peptide (NT-proBNP) are predictors of prognosis in patients with coronary artery disease (CAD). We aimed at evaluating the effect of coronary atherosclerosis and myocardial ischemia on cardiac release of hs-cTnT and NT-proBNP in patients with suspected CAD.

Approach and Results—Hs-cTnT and NT-proBNP were measured in 378 patients (60.1±0.5 years, 229 males) with stable angina and unknown CAD enrolled in the Evaluation of Integrated Cardiac Imaging (EVINCI) study. All patients underwent stress imaging to detect myocardial ischemia and coronary computed tomographic angiography to assess the presence and characteristics of CAD. An individual computed tomographic angiography score was calculated combining extent, severity, composition, and location of plaques. In the whole population, the median (25–75 percentiles) value of plasma hs-cTnT was 6.17 (4.2–9.1) ng/L and of NT-proBNP was 61.66 (31.2–132.6) ng/L. In a multivariate model, computed tomographic angiography score was an independent predictor of the plasma hs-cTnT (coefficient 0.06, SE 0.02; P=0.0089), whereas ischemia was a predictor of NT-proBNP (coefficient 0.38, SE 0.12; P=0.0015). Hs-cTnT concentrations were significantly increased in patients with CAD with or without myocardial ischemia (P<0.005), whereas only patients with CAD and ischemia showed significantly higher levels of NT-proBNP (P<0.001).

Conclusions—In patients with stable angina, the presence and extent of coronary atherosclerosis is related with circulating levels of hs-cTnT, also in the absence of ischemia, suggesting an ischemia-independent mechanism of hs-cTnT release. Obstructive CAD causing myocardial ischemia is associated with increased levels of NT-proBNP. **(*Arterioscler Thromb Vasc Biol*. 2016;36:757-764. DOI: 10.1161/ATVBAHA.115.306818.)**

Key Words: coronary artery disease ■ hs cardiac troponin T ■ myocardial ischemia ■ NT-proBNP ■ stable angina

Patients with stable coronary artery disease (CAD) represent a heterogeneous group in terms of their pathophysiological substrate, clinical presentation, and outcome. They may have different morphology, severity, and extent of coronary atherosclerotic disease, as well as a different prevalence of myocardial ischemia. These multiple aspects may have independent prognostic value.[1]

High-sensitivity cardiac troponins (hs-cTn) are commonly used in the diagnosis of acute coronary syndromes.[2] Recently, it has been shown that episodes of minute troponin release, below the threshold for acute myocardial infarction, often occur in patients with stable CAD and predict all-cause mortality, cardiovascular mortality, and heart failure (HF).[3–5] N-terminal pro brain natriuretic peptide (NT-proBNP) is a powerful prognostic indicator in patients with left ventricular (LV) dysfunction and HF. However, it is also a predictor of all-cause and cardiovascular mortality in patients with stable CAD without HF.[5–7] It has been shown that combined use of hs-cTnT and NT-proBNP improves long-term risk prediction of mortality in patients with stable CAD.[5] Hence, it is conceivable that myocardial release of hs-cTnT or NT-proBNP might be linked to

Received on: November 3, 2015; final version accepted on: February 1, 2016.

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The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.115.306818/-/DC1.

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Nonstandard Abbreviations and Acronyms	
CAD	coronary artery disease
CTA	computed tomographic angiography
EVINCI	Evaluation of Integrated Cardiac Imaging
HF	heart failure
hs-cTnT	high-sensitivity cardiac troponin T
LV	left ventricle
NT-proBNP	N-terminal pro brain natriuretic peptide

different coronary pathophysiological substrates with additive impact on prognosis.

The aim of this study was to evaluate the combined effect of coronary atherosclerosis, assessed by computed tomographic angiography (CTA), and myocardial ischemia, assessed by stress imaging, on cardiac release of hs-cTnT and NT-proBNP in patients with stable CAD enrolled in the Evaluation of Integrated Cardiac Imaging (EVINCI) study.[8]

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Baseline Clinical Characteristics of Study Population

In the entire population (Figure 1), the median value (25–75 percentile) of plasma hs-cTnT was 6.17 (4.16–9.09) ng/L and of NT-proBNP was 61.66 (31.19–132.60) ng/L.

Only 34 (9%) and 77 (20%) patients had plasma levels of hs-cTnT and NT-proBNP exceeding the upper limits of the normality ranges (14 and 157 ng/L, respectively). The median values were used as cut-off points to divide patients into groups with low (<median) or high (≥median) concentrations of hs-cTnT and NT-proBNP. Baseline characteristics of the patients are compared between groups in Table 1. Patients with high levels of hs-cTnT were older and had higher frequency of male sex, diabetes mellitus, and hypertension than patients with low levels of hs-cTnT. Patients with high levels of NT-proBNP were also older than patients with low levels of NT-proBNP but showed a lower frequency of male sex and diabetes mellitus and a lower LV ejection fraction (LVEF). No differences in type of symptoms were observed among patients with high or low hs-cTnT or NT-proBNP. The metabolic and inflammatory profile was altered in patients with high levels of hs-cTnT but not in those with high NT-proBNP levels.

Coronary Atherosclerosis and Myocardial Ischemia

Results from CTA (coronary anatomy, plaque characterization, and risk scores) and stress imaging (myocardial ischemia) are compared between groups of patients with high or low hs-cTnT or NT-proBNP in Table 2.

Biomarker levels were associated with the severity and the number of coronary lesions. Patients with high hs-cTnT as well as patients with high NT-proBNP showed a higher frequency of obstructive coronary lesions as compared with patients with low hs-cTnT and NT-proBNP who showed a higher frequency of normal coronary arteries. Figure 2 illustrates the increase of hs-cTnT and NT-proBNP plasma levels according to the presence of nonobstructive or obstructive coronary lesions (Figure 2A) or the number of coronary lesions (either obstructive or nonobstructive; Figure 2B).

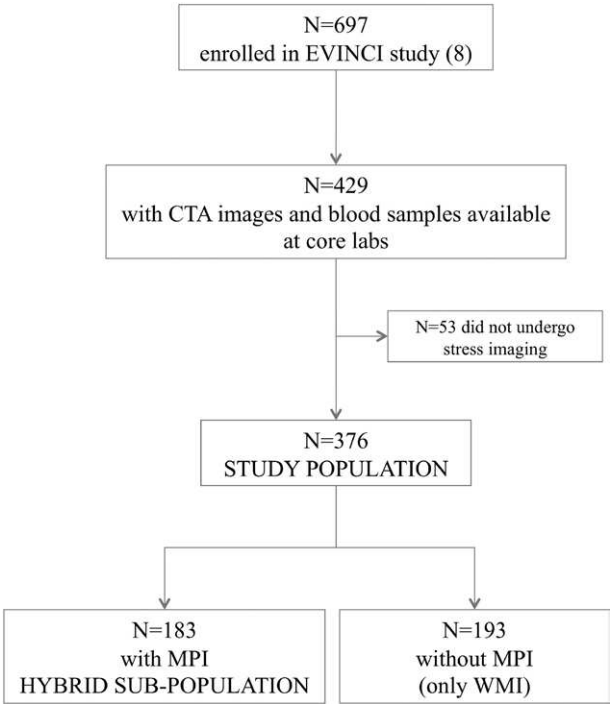

Figure 1. Patient flow chart. CTA indicates computed tomographic angiography; EVINCI, Evaluation of Integrated Cardiac Imaging; MPI, myocardial perfusion imaging; and WMI, wall motion imaging.

The 2 biomarkers were differently associated with plaque types. Patients with high hs-cTnT, but not patients with high NT-proBNP, showed more frequently calcified or mixed plaques than patients with low hs-cTnT and had a higher number of mixed plaques. The CTA risk score was significantly higher in patients with high hs-cTnT or high NT-proBNP, whereas coronary artery calcium score was significantly higher only in patients with high hs-cTnT (Table 2).

Both patients with high hs-cTnT and high NT-proBNP had a higher frequency and larger extent of myocardial ischemia (Table 2).

Integrated Effects of Coronary Atherosclerotic Burden and Myocardial Ischemia on Circulating hs-cTnT and NT-proBNP Levels and LV Function

Univariate (Table I in the online-only Data Supplement) and multivariate linear regression models were used to identify the independent predictors of elevated levels of hs-cTnT and NT-proBNP (Table 3). Age, male sex, and CTA risk score were independent predictors of increased hs-cTnT levels. Age, female sex, lower LVEF, and the presence of ischemia were independent predictors of increased NT-proBNP levels.

Table 1. Baseline Clinical and Biohumoral Characteristics

Clinical Variables	Low hs-cTnT (n=188)	High hs-cTnT (n=188)	*P* Value	Low NT-proBNP (n=188)	High NT-proBNP (n=188)	*P* Value
Demographics						
Age, y	57.7±0.6	62.5±0.6	<0.0001	57.4±0.7	62.8±0.6	<0.0001
Male sex	95 (42)	131 (58)	0.0001	128 (56.6)	98 (43.4)	0.0016
CV risk factors						
Family history of CAD	70 (45.3)	58 (54.7)	ns	70 (54.7)	58 (45.3)	ns
Diabetes mellitus	30 (33.7)	59 (66.3)	0.0004	53 (59.5)	36 (40.5)	0.0392
Hypertension	101 (45.9)	124 (55.1)	0.0155	107 (47.6)	118 (52.4)	ns
Hypercholesterolemia	116 (53)	103 (47)	ns	111 (50.7)	108 (49.3)	ns
Obesity	35 (44.9)	43 (55.1)	ns	38 (48.7)	40 (51.3)	ns
Smoking	48 (55.3)	42 (46.7)	ns	49 (54.4)	41 (45.6)	ns
Symptoms						
Typical angina	47 (54.6)	39 (45.4)		39 (45.3)	47 (54.7)	
Atypical angina	118 (50)	118 (50)		124 (52.5)	112 (47.6)	
Nonanginal chest pain	23 (42.6)	31 (57.4)	ns	25 (46.3)	29 (53.7)	ns
LV Function						
LVEF, %	60 [55–67]	60 [55–65]	ns	62 [58.5–67]	60 [50–65]	<0.0001
Medications						
Beta-blockers	74 (49.3)	76 (50.7)	ns	51 (34)	99 (66)	<0.0001
Calcium antagonists	17 (38.6)	27 (61.4)	ns	19 (43.2)	25 (56.8)	ns
ARBs/ACE Inhibitors	60 (37.7)	99 (62.3)	<0.0001	72 (42.3)	87 (54.7)	ns
Diuretics	25 (39)	39 (61)	ns	31 (48.4)	33 (51.6)	ns
Nitrates	16 (43.2)	21 (56.8)	ns	16 (43.2)	21 (50.8)	ns
Antithrombotics	104 (46.4)	120 (53.6)	ns	100 (44.6)	124 (55.4)	0.0117
Oral antidiabetics/Insulin	23 (31.5)	50 (68.5)	0.0004	40 (54.8)	33 (45.2)	ns
Statins	99 (50.3)	98 (49.7)	ns	93 (44.7)	104 (55.3)	ns
Bio-humoral variables						
Creatinine, mg/dL	0.82 [0.68–0.97]	0.86 [0.75–1.02]	0.0113	0.85 [0.72–1.00]	0.83 [0.73–0.98]	ns
Glucose, mg/dL	101 [90.8–114]	105.0 [93–126]	0.0033	104 [102–120]	102 [92–115.3]	ns
Total cholesterol, mg/dL	183 [147.3–220]	177 [142.3–209.5]	ns	185 [147.3–216]	171 [142–214.8]	ns
LDL cholesterol, mg/dL	103 [78–136]	99 [75–128]	ns	107 [78–131.5]	96 [75–128.2]	ns
HDL cholesterol, mg/dL	52 [41–65]	47 [39.3–58]	0.0087	49 [41–59]	50 [40–63]	ns
Triglycerides, mg/dL	98 [70.3–147.5]	109 [77.3–156]	0.0440	113 [77.3–163]	98 [71.3–128.5]	0.0048
hs-CRP, mg/dL	0.13 [0.07–0.30]	0.19 [0.09–0.38]	0.0427	0.14 [0.07–0.36]	0.18 [0.09–0.34]	ns
hs-cTnT, ng/L	4.2 [3.0–5.2]	9.09 [7.3–12.6]	<0.0001	5.67 [4.0–8.3]	6.75 [4.5–9.6]	0.0122
NT-proBNP, ng/L	55.3 [28.05–106.3]	68.0 [36.5–168.3]	<0.0001	31.2 [16.1–49.1]	132.6 [87.2–206.4]	<0.0001

Continuous variables are presented as mean±standard error or median [25–75 percentile], categorical variables as absolute N and (%). ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blockers; CAD, coronary artery disease; CV, cardiovascular; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; LDL, low-density lipoprotein; LV, left ventricle; LVEF, left ventricular ejection fraction; and NT-proBNP, N terminal pro brain natriuretic peptide.

In a sex- and age-specific analysis, CTA risk score was significantly related with hs-cTnT levels in both men and women >60 years, whereas the presence of ischemia was related with increased NT-proBNP in males independently of age (Tables II and III in the online-only Data Supplement).

Patients were subdivided into groups according to the absence/presence of coronary atherosclerosis (CAD) and ischemia, either alone or combined. Patients with CAD with/without ischemia showed significantly higher levels of hs-cTnT than patients without CAD, whereas only patients with CAD plus ischemia had significantly higher levels of NT-proBNP as compared with all the other groups (Figure 3A).

Similar results were obtained by hybrid imaging analysis. Patients with an inducible perfusion defect in a territory subtended by a stenotic coronary artery on CTA (matched) were compared with patients with all other combinations of pathological findings (unmatched) and with normals. As compared with normals, NT-proBNP levels were significantly increased only in patients with matched findings, whereas hs-cTnT levels were significantly elevated in both patients with matched and mismatched findings (Figure 3B).

Table 2. Coronary Imaging and Myocardial Ischemia

	Low hs-cTnT (n=188)	High hs-cTnT (n=188)	*P* Value	Low NT-proBNP (n=188)	High NT-proBNP (n=188)	*P* Value
Coronary anatomy						
Normals	80 (71.4)	32 (28.6)	<0.0001	67 (59.8)	45 (40.2)	0.0004
Patients with nonobstructive CAD (<50%)	65 (46.1)	76 (53.9)		77 (54.6)	64 (45.4)	
Patients with obstructive CAD (≥50%)	43 (35)	80 (65)		44 (35.8)	79 (64.2)	
Coronary plaques						
Patients with calcified plaque	57 (41.9)	79 (58.1)	0.0182	66 (49.1)	70 (50.1)	ns
Patients with mixed plaque	87 (39.2)	135 (60.8)	<0.0001	103 (46.4)	119 (53.6)	ns
Patients with noncalcified plaque	47 (44.3)	59 (55.7)	ns	52 (49.1)	54 (50.9)	ns
No of calcified plaques	0.7±0.12	1±0.13	ns	0.9±0.1	0.8±0.1	ns
No of mixed plaques	1.7±0.19	3.3±0.25	<0.0001	2.2±0.22	2.8±0.25	ns
No of noncalcified plaques	0.4±0.06	0.5±0.07	ns	0.4±0.06	0.5±0.07	ns
Risk scores						
CTA risk score	4.0 [0.0–15.0]	12.9 [5.6–23.1]	<0.0001	7.2 [0.0–16.6]	11.8 [1.7–22.3]	0.0084
CAC score (n= 297)	6 [0–127]	78 [6–423]	<0.0001	31 [0–202]	46 [0–273]	ns
Myocardial ischemia						
Patients with myocardial ischemia (any modality)	37 (37.7)	61 (62.3)	0.0046	34 (34.7)	64 (65.3)	0.0004
Patients with myocardial ischemia (MPI, n=193)	33 (37.1)	56 (62.9)	0.0209	29 (32.6)	60 (67.4)	0.0003
SDS at MPI (n=193)	2.76±0.57	4.10±0.65	0.0274	1.79±0.39	5.06±0.74	<0.0001

Continuous variables are presented as mean±standard error or median [25–75 percentile], categorical variables as absolute N and (%). CAC indicates coronary artery calcium; CAD, coronary artery disease; CTA, computed tomographic angiography; CV, cardiovascular; hs-cTnT, high-sensitivity cardiac troponin T; MPI, myocardial perfusion imaging; NT-proBNP, N terminal pro brain natriuretic peptide; and SDS, summed difference scores.

LVEF and NT-proBNP were linearly correlated in the whole population (*P*=0.002). Interestingly, only patients with coronary atherosclerosis and myocardial ischemia had significantly decreased LVEF values as compared with all the other patient groups (Figure 4).

Discussion

This is the first study which evaluated the relative effects of both coronary atherosclerosis and myocardial ischemia on cardiac release of hs-cTnT and NT-proBNP in patients with stable CAD. The main findings can be summarized as follows:

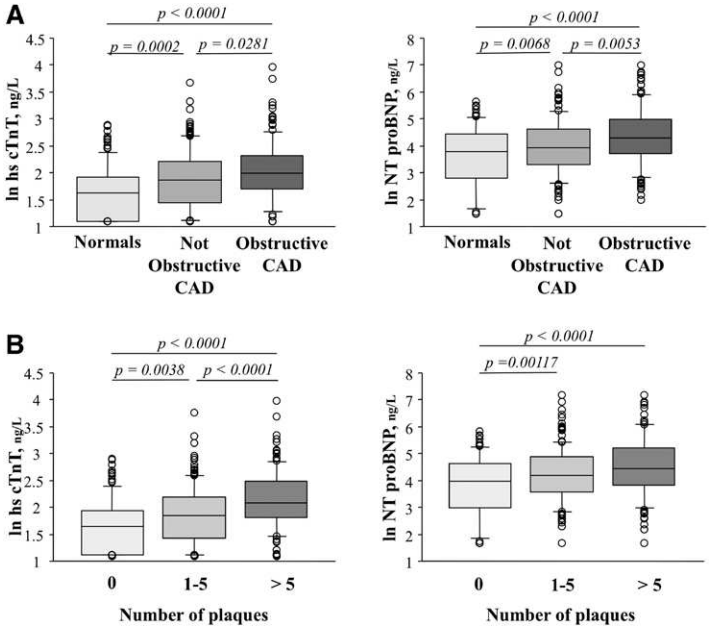

Figure 2. Relation of high-sensitivity cardiac troponin T (hs-cTnT) and N terminal pro brain natriuretic peptide (NT-proBNP) with severity and extent of coronary artery disease (CAD). Box plots represent hs-cTnT and NT-proBNP levels stratified per CAD severity (**A**) and number of plaques (**B**).

Table 3. Predictive Factors of hs-cTnT and NT-proBNP Plasma Levels at Multivariate Analyses

	hs-cTnT		NT-proBNP	
Variables	Coefficient (SE)	*P* Value	Coefficient (SE)	*P* Value
Age	0.012 (0.003)	0.0007	0.041 (0.006)	<0.0001
Sex	0.282 (0.057)	<0.0001	−0.258 (0.104)	0.0132
LVEF	…	…	−0.880 (0.348)	0.0119
CTA risk score	0.062 (0.023)	0.0068	…	…
Presence of Ischemia	…	…	0.369 (0.114)	0.0013

Models were adjusted for variables significantly associated with biomarker levels at multivariate analysis, including oral antidiabetics/insulin and high-sensitivity C-reactive protein for hs-cTnT and β-blockers and tryglicerides for NT-proBNP. CTA indicates computed tomographic angiography; hs-cTnT, high-sensitivity cardiac troponin T; LVEF, left ventricular ejection fraction; and NT-proBNP, N terminal pro brain natriuretic peptide.

1. Circulating levels of hs-cTnT are related to the presence and extent of coronary lesions as well as coronary plaque composition; in addition, the global coronary atherosclerotic burden, as assessed by the CTA risk score, is a predictor of hs-cTnT plasma levels independent of myocardial ischemia.
2. Circulating levels of NT-proBNP are mainly related with the presence of functionally relevant coronary disease causing myocardial ischemia.

Cardiac troponins are the marker of choice for the detection of myocardial injury and the diagnosis of myocardial infarction.[2] Over the past 10 years, cardiac troponin assays have been improved in analytic sensitivity and precision, thereby allowing the measurement of cardiac troponin in nearly all healthy subjects.[9] Because of the significant biological variability of this biomarker, individual measurements can be more relevant than population reference values.[10] In stable CAD, the release of cardiac troponin T has been linked to the extent of coronary atherosclerosis,[3–5,11] although the relationship with transient myocardial ischemia is debated.[12,13] In the current study, only the global atherosclerotic burden, as assessed by the CTA risk score, was an independent predictor of hs-cTnT levels at multivariate analysis. This association was specifically evident in older patients, independently of sex. Patients with coronary atherosclerosis showed significantly higher levels of hs-cTnT compared with patients without, independent of the presence of myocardial ischemia (Figure 3).

The mechanisms of hs-cTnT elevation in patients with stable CAD, who had no occurrence of acute events with myocyte necrosis, are not fully understood. Consistently with the present findings, it has been hypothesized that at the site of atherosclerotic lesions, dislodging and erosion processes could cause microembolization of atherosclerotic and thrombotic material into the microcirculation.[11,14] It has been reported that chronic clinically silent rupture of noncalcified plaques with subsequent microembolization may be a potential source of troponin elevation.[15] These findings are in line with the results from the present study, in which the patients with higher coronary atherosclerotic burden and mixed plaques showed higher levels of hs-cTnT than the patients without. Mechanisms which link microembolization with myocardial damage and troponin release involve tumor necrosis factor-α and nitric oxide, which may trigger apoptotic pathways of cardiomyocytes.[16] During the apoptotic process, caspase-3 activation results in cleavage of cTn and subsequent release.[11] Different mechanisms explaining how cardiomyocytes could release cTnT have been suggested,[12] including membranous blebs enabling troponin to be released from cardiac cells because of ischemia alone without necrosis.[17]

Elevation of circulating NT-proBNP is proportional to the extent of ventricular dysfunction in patients with or without overt HF.[18] There are few data demonstrating the association of NT-proBNP levels with both coronary

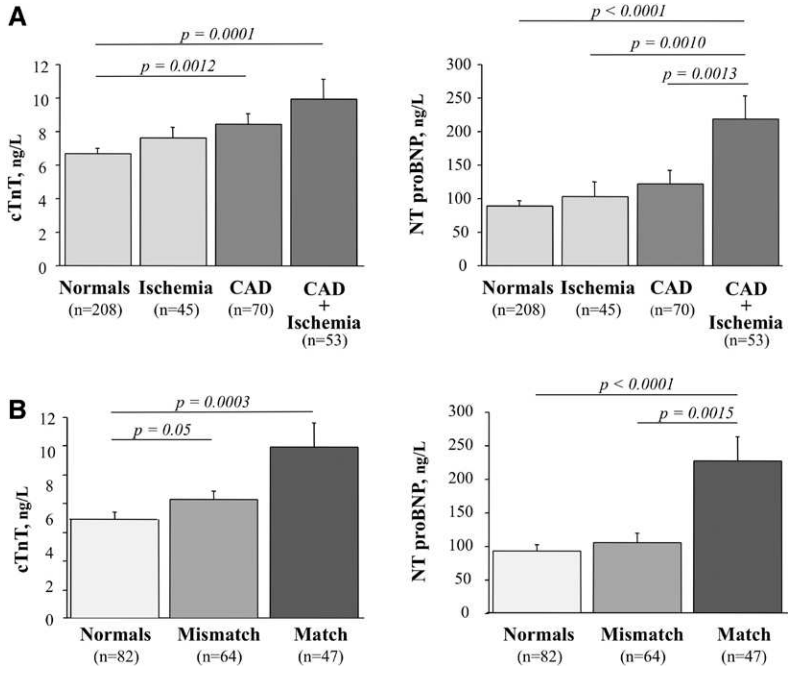

Figure 3. Relative effects of obstructive coronary stenoses and myocardial ischemia on high-sensitivity cardiac troponin T (hs-cTnT) and N terminal pro brain natriuretic peptide (NT-proBNP) plasma levels. Circulating levels of hs-cTnT and NT-proBNP are presented in patients subdivided according to (**A**) the absence of both obstructive coronary stenoses and ischemia (Normals), the presence of myocardial ischemia without obstructive coronary stenoses (Ischemia), the presence of obstructive coronary stenoses without myocardial ischemia (coronary artery disease [CAD]), and the presence of both (CAD+Ischemia) at separate analysis of computed tomographic angiography (CTA) and stress imaging; and (**B**) the absence or the presence of matched and unmatched findings at hybrid imaging analysis of CTA and myocardial perfusion imaging (MPI).

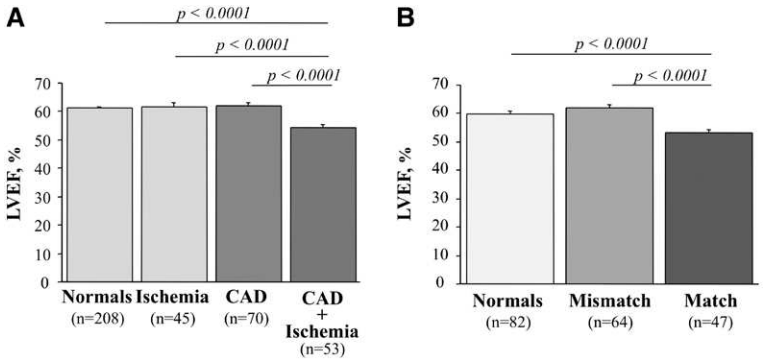

Figure 4. Effects of obstructive coronary stenoses and myocardial ischemia on left ventricular (LV) systolic function. LV ejection fraction (LVEF) in patients subdivided into groups according to the absence/presence of obstructive coronary artery disease (CAD) and myocardial ischemia alone or in combination (**A**) and in patients with matched and unmatched findings at hybrid imaging analysis (**B**), as in Figure 2.

atherosclerosis and myocardial ischemia in patients with stable CAD.[19,20] Experimental and clinical studies[21,22] have provided evidence that myocardial ischemia may cause per se an increased expression and secretion of natriuretic peptides by ventricular myocytes. In this study, both coronary atherosclerotic burden and presence of myocardial ischemia were predictors of higher NT-proBNP plasma levels in patients with stable CAD. However, in contrast to hs-cTnT, only the presence of myocardial ischemia was an independent predictor of elevated NT-proBNP. This association was mainly evident in males who had a higher prevalence of obstructive CAD and severe myocardial ischemia (Table IV

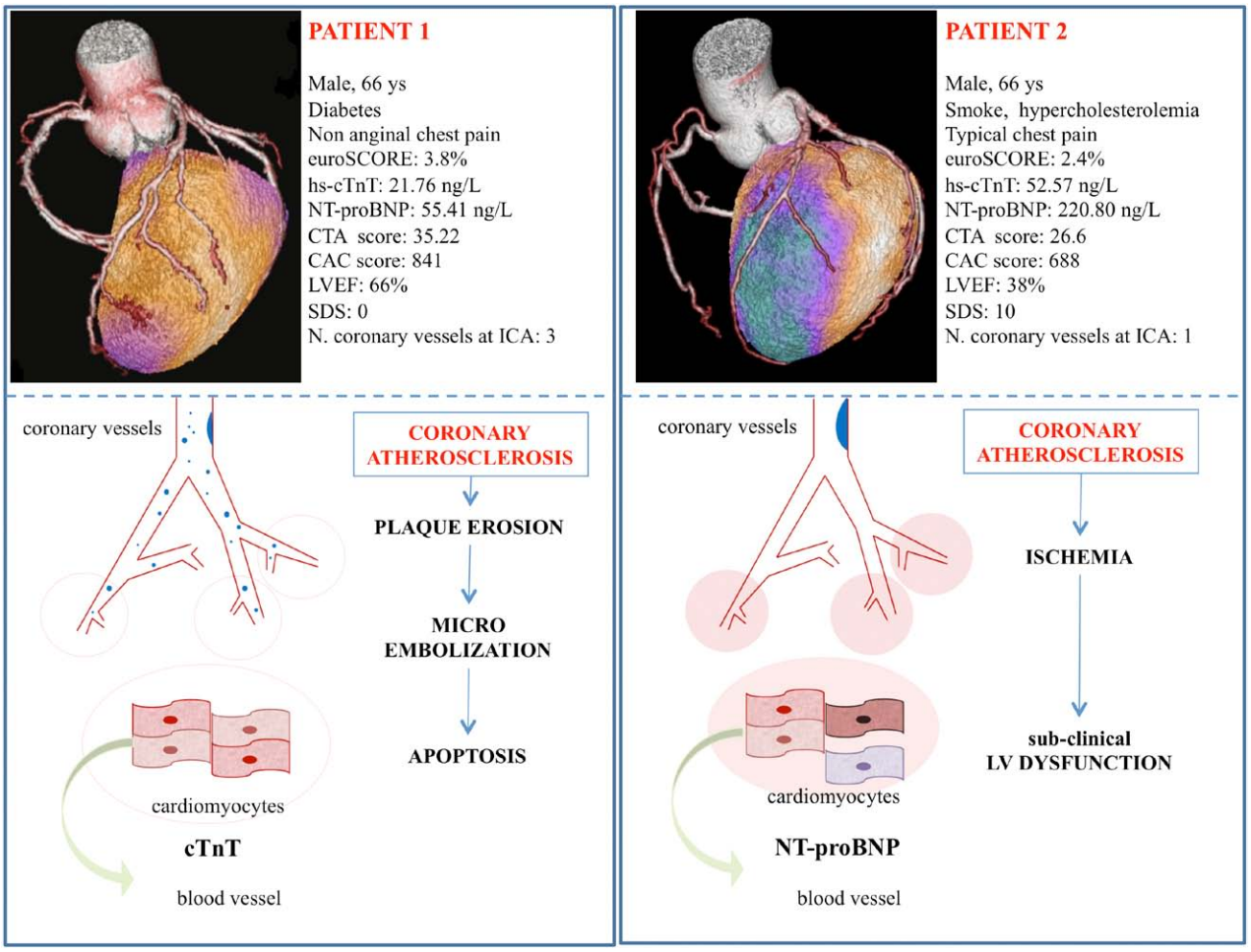

Figure 5. Schematic model illustrating the possible mechanisms explaining the relationships between high-sensitivity cardiac troponin T (hs-cTnT) and N terminal pro brain natriuretic peptide (NT-proBNP) plasma levels and coronary disease in 2 representative patients with stable angina. Hybrid images of 2 cases are reported. Patients 1: at the site of the atherosclerotic lesions, dislodging and erosion processes could cause microembolization of atherosclerotic and thrombotic material into the microcirculation. This phenomenon may trigger the apoptotic process with consequent release of hs-cTnT from cardiac myocytes into peripheral circulation. Patients 2: the presence of obstructive coronary disease associated with myocardial ischemia may increase circulating levels of NT-proBNP by its augmented release from ischemic cardiac myocytes. CAC indicates coronary artery calcium; CTA, computed tomographic angiography; and LVEF, left ventricular ejection fraction.

in the online-only Data Supplement). Patients with obstructive coronary lesions and ischemia had significantly higher values of NT-proBNP than all other patients (Figure 3). Interestingly, this subgroup of patients had also significantly decreased LVEF values as compared with all other patient groups (Figure 4). Increased release of brain natriuretic peptide may be consequent to the effects of chronic repetitive ischemia on the myocardium, including both a direct stimulation of natriuretic peptide gene expression in the myocytes[22] and a mechanical stimulation induced by subclinical myocardial dysfunction with higher wall stress.[23] Independent of the mechanism, the current findings underline the relevance of circulating NT-proBNP as a marker of chronic ischemic coronary disease and are in agreement with the known prognostic role of this biomarker in patients with stable CAD.[6,7]

A schematic representation of the proposed mechanisms explaining the increased levels of hs-cTnT and NT-proBNP is reported in Figure 5 together with data from 2 representative patients with stable CAD.

Limitations

Assessment of ischemia was performed with varying modalities (positron emission tomography, single-photon emission computed tomography, stress echocardiography, or cardiac magnetic resonance imaging), which is a limitation but reflects the clinical reality (not every center applies the same diagnostic tests). Similarly, the definition of ischemic abnormalities included resting myocardial perfusion or wall motion defects compatible to some degree of scar. However, the main study findings were confirmed in the 193 patients with myocardial perfusion imaging and hybrid imaging where only inducible myocardial ischemia downstream a significant coronary lesion was considered.

The EVINCI protocol did not require evaluation of LV diastolic function. It cannot be excluded that some patients with multiple risk factors (hypertension, diabetes mellitus, obesity) had unrecognized heart failure with preserved ejection fraction and that this could have somewhat influenced NT-proBNP and hs-cTnT levels.[24,25]

In addition, another limitation includes that other troponin isoforms or degradation products[26] as well as other active BNP peptides[27] could have been measured extending our present understanding of the effects of the different patterns of atherosclerotic disease on both cardiac structure and endocrine function.

Conclusions

The present study provides evidence that circulating hs-cTnT and NT-proBNP levels, even if in the normal range, may provide different and complementary information on the presence and severity of CAD in patients with stable angina. Relative increase in circulating hs-cTnT is a marker of the coronary atherosclerotic process being related with the global atherosclerotic burden and with the type of atherosclerotic plaques. On the contrary, relative increase in circulating NT-proBNP is mainly associated with myocardial ischemia because of more severe coronary lesions and expresses a subclinical LV dysfunction possibly consequent to the chronic ischemic process. According to the present results, hs-cTnT and NT-proBNP may have a relevant clinical role in the screening process and in the prognostic stratification of patients with suspected CAD. Further studies are needed to assess whether these biomarkers can improve the accuracy of current diagnostic and prognostic predictive models and help to target personalized management in patients with stable angina.

Acknowledgments

Acknowledgments to Roche Diagnostics International (Roche Diagnostics International Ltd, Switzerland) that supplied all reagents and calibrators for the measurement of hs-cTnT and NT-proBNP plasma levels in the EVINCI population and to Dr Daniela Bellavista (Roche Diagnostics, Italy) for her valuable local support.

Sources of Funding

This work was supported by a grant from the European Union FP7-CP-FP506 2007 project (grant agreement no 222915, EVINCI).

Disclosures

None.

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Significance

Patients with stable coronary artery disease (CAD) represent a heterogeneous group in terms of their pathophysiological substrate, clinical presentation, and outcome. Circulating levels of high-sensitivity cardiac troponin T (hs-cTnT) and N terminal pro brain natriuretic peptide (NT-proBNP) are independent predictors of prognosis in patients with CAD. This is the first study which evaluated the relative effects of both coronary atherosclerosis and myocardial ischemia on cardiac release of hs-cTnT and NT-proBNP in patients with stable CAD, thus providing evidence that circulating hs-cTnT and NT-proBNP may provide different and complementary information on presence and severity of CAD. In particular, the features of coronary atherosclerosis are related with circulating hs-cTnT, also in the absence of ischemia; obstructive CAD causing myocardial ischemia is associated with increased levels of NT-proBNP. Accordingly, hs-cTnT and NT-proBNP may have a relevant clinical role in the screening process and in the prognostic stratification of patients with suspected CAD.

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Effect of Coronary Atherosclerosis and Myocardial Ischemia on Plasma Levels of High-Sensitivity Troponin T and NT-proBNP in Patients With Stable Angina

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Arterioscler Thromb Vasc Biol. 2016;36:757-764; originally published online February 11, 2016;
doi: 10.1161/ATVBAHA.115.306818
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636

Table I Supplementary material. Relationship between clinical, bio-humoral variables and imaging results with hs-cTnT and NT-proBNP at univariate analysis.

Variables	hs-cTnT		NT-proBNP	
Demographics	***Coefficient (SE)***	***P value***	***Coefficient (SE)***	***P value***
Age—yr	0.015 (0.003)	<0.0001	0.053 (0.006)	<0.0001
Male sex, n (%)	0.301 (0.057)	<0.0001	- 0.401 (0.116)	0.0006
CV risk factor				
Family history of CAD	- 0.065 (0.061)	0.2870	- 0.188 (0.121)	0.1213
Diabetes mellitus	0.290 (0.066)	<0.0001	- 0.092 (0.135)	0.4976
Hypertension	0.150 (0.058)	0.0104	0.291 (0.117)	0.0129
Hypercholesterolemia	- 0.119 (0.058)	0.0407	- 0.063 (0.117)	0.5925
Obesity	- 0.059 (0.071)	0.4029	- 0.014 (0.142)	0.9207
Smoking (last year)	0.006 (0.067)	0.9317	- 0.134 (0.135)	0.3218
LV function				
LVEF, %	- 0.234 (0.199)	0.2422	- 1.497 (0.393)	0.0002
Medications				
Beta-blockers	0.013 (0.059)	0.8226	0.816 (0.110)	<0.0001
Calcium antagonist	0.076 (0.089)	0.3963	0.118 (0.179)	0.511
ARBs/ACE Inhibitors	0.214 (0.057)	0.0002	0.335 (0.115)	0.0039
Diuretics	0.112 (0.076)	0.1415	0.195 (0.153)	0.2040
Nitrates	0.012 (0.096)	0.9029	0.172 (0.193)	0.3740
Anti-thrombotics	0.080 (0.058)	0.1688	0.406 (0.115)	0.0005
Oral antidiabetics/Insulin	0.363 (0.070)	<0.0001	0.119 (0.146)	0.4148
Statins	0.005 (0.057)	0.9291	0.227 (0.115)	0.0484
Bio-humoral markers				
Creatinine	0.437 (0.119)	0.0003	- 0.036 (0.243)	0.8812
Glucose	0.413 (0.144)	0.0003	- 0.039 (0.233)	0.8657
Total cholesterol	- 0.198 (0.170)	0.0648	- 0.608 (0.213)	0.0046
LDL cholesterol	- 0.102 (0.072)	0.1578	- 0.330 (0.144)	0.0227
HDL cholesterol	- 0.274 (0.091)	0.0028	- 0.0.84 (0.185)	0.6504
Triglycerides	0.088 (0.053)	0.0989	- 0.344 (0.105)	0.0012
hs CRP	0.046 (0.026)	0.07861	0.048 (0.053)	0.3603
Imaging results				
CTA Risk Score	0.134 (0.020)	<0.0001	0.208 (0.042)	<0.0001
Presence of ischemia	0.168 (0.065)	0.0098	0.531 (0.128)	<0.0001

Table II Supplementary material. Relationship between hs-cTnT and CTA risk score in patient groups subdivided according to sex and age.

		hs cTnT					
		Males			Females		
		n	*Coefficient (SE)*	*P value*	*n*	*Coefficient (SE)*	*P value*
CTA Risk Score	**Age < 60 ys**	112	0.038 (0.04)	---	49	0.128 (0.09)	---
	Age ≥ 60 ys	114	0.147 (0.06)	0.0121	101	0.113 (0.04)	0.0012

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Table III Supplementary material. Relationship between NT-proBNP and Presence of Ischemia in patient groups subdivided according to sex and age.

		NT-proBNP					
		Males			Females		
		n	*Coefficient (SE)*	*P value*	*n*	*Coefficient (SE)*	*P value*
Presence of Ischemia	**Age < 60 ys**	112	0.798 (0.22)	0.0004	49	0.022 (0.52)	---
	Age ≥ 60 ys	114	0.713 (0.21)	0.0009	101	0.022 (0.22)	---

Table IV Supplementary material. Coronary anatomy and myocardial ischemia according to gender.

	Males (n=226)	Females (n=150)	P value
Coronary anatomy			
Normals	49 (43.7)	63 (56.3)	
Patients with non-obstructive CAD (<50%)	93 (66)	48 (34)	0.0001
Patients with obstructive CAD (≥50%)	84 (68.3)	39 (31.7)	
Myocardial ischemia			
Patients with myocardial ischemia (any modality)	71 (72.5)	27 (27.5)	0.0037
Patients with myocardial ischemia (MPI, n=193)	66 (74.2)	23 (25.8)	0.0050
SDS at MPI (n=193)	3.97 ± 1.15	1.54 ± 1.50	0.0166

Continuous variables are presented as mean ± standard error; categorical variables as absolute N and (%).

Materials and Methods

Population, diagnostic protocol and study design

Patients with stable chest pain or equivalent symptoms and intermediate probability of CAD were studied. These patients were enrolled at 14 European centres in the EVINCI study (1). The study protocol is available at http://www.clinicaltrials.gov (NCT00979199). Briefly, patients with acute coronary syndrome, known CAD, left ventricular ejection fraction < 35%, significant heart valve disease, cardio-myopathy or contraindications to stress imaging were excluded. According to the protocol, each patient underwent CTA, stress imaging by myocardial perfusion imaging (MPI) or wall motion imaging (WMI). If at least one non invasive test was positive, invasive coronary angiography, with fractional flow reserve (FFR) assessment if indicated, was performed. Ethical approval was provided by each participating centre and all subjects provided written informed consent.

The patients whose CTA images, stress images and plasma samples were available for core laboratory analyses were included in this study. In a subgroup of patients image fusion of CTA datasets with single-photon emission computed tomography (SPECT) or positron emission tomography (PET) was obtained and hybrid analysis performed by a dedicated core laboratory (Figure 1).

Blood collection and analysis

Blood samples were collected prior to imaging in EDTA tubes and then locally separated by centrifugation for 15 min at 1000 ×g. Refrigerated plasma samples were shipped to the bio-humoral core laboratory (CNR-Institute of Clinical Physiology, Pisa, Italy) for the final cryo-conservation in the EVINCI biological bank (2-3). Analyses of hs-cTnT and NT-proBNP were performed at the Laboratory of Fondazione Toscana G. Monasterio (Pisa, Italy). Plasma concentrations of cTnT were measured using the hs-cTnT method on COBAS E411 with Elecsys Troponin T hs STAT by Roche Diagnostics (4). Measurement of NT pro-BNP was performed using the electrochemical luminescence immunoassay Elecsys proBNP II by Roche Diagnostics using monoclonal antibodies (5). In order to complete the clinical/biohumoral profile of the study patients, additional traditional biomarkers were measured using standard methods (2-3).

Image acquisition

Image acquisition protocols were agreed on for each technique covering patient preparation, cardiovascular stress, administration of radiopharmaceutical or contrast medium, image acquisition and quality control. These procedures were based on best available clinical practice. Image reading was performed at core labs for each specific technique (1).

Coronary CTA analysis and CTA risk score

The coronary CTAs were analyzed in a core laboratory (Leiden University Medical Center, Leiden, The Netherlands) as described elsewhere (1-2). Briefly, each segment of the AHA 17-coronary segment model was assessed for interpretability, and interpretable segments were evaluated for stenosis of the coronary artery lumen providing three different categories: normal if no atherosclerosis was present, non-obstructive if the stenosis severity was <50%, and obstructive for stenoses >50%. If plaque was present, plaque composition was visually determined (calcified, mixed, and non-calcified). Only one type of plaque composition could be assigned to a single segment.

A previously established CTA risk score was derived in each patient integrating all data on the location, severity, and composition of CAD (2, 6). This risk score was used as an indicator of the global coronary atherosclerotic burden at individual level similarly to the synthetic scores used to describe the extent of coronary disease from invasive angiography (7). In 297/376 patients, CT acquisitions for coronary artery calcium (CAC) were available and the Agatston CAC score was computed according to standard methods.

Non invasive stress imaging analysis

MPI and WMI were defined as abnormal if there was either an inducible perfusion abnormality or myocardial scarring. Perfusion in each of 17 myocardial segments was classified as 0=normal, 1=mild reduction, 2=moderate reduction, 3=severe reduction or 4=absent perfusion and the segmental scores were summed for the stress and rest images. For MPI, an inducible perfusion abnormality (ischemia) was defined as a summed segmental difference score between stress and rest images ≥ 2, either from a score ≥ 1 in at least two contiguous segments or ≥ 2 in at least one segment. Scarring was defined similarly from the summed segmental rest score. For WMI, segmental myocardial wall motion was scored at rest and during stress as normal (0), hypokinetic (1), akinetic (2) or dyskinetic (3). Inducible ischemia was defined as an increase in segmental wall motion score ≥ 1 from rest to stress in at least two contiguous segments. Scarring was defined similarly from the resting wall motion score.

Hybrid imaging

In the subgroup of 193 patients submitted to MPI by PET or SPECT, a hybrid imaging study was performed. Individual datasets from MPI and CTA were transferred to a dedicated hybrid core laboratory (University Hospital Zurich, Switzerland). Image fusion of MPI /CTA datasets was performed on a dedicated workstation (Advantage Workstation 4.4, GE Healthcare) using the CardIQ Fusion software (GE Healthcare) (8).

All hybrid MPI/CTA images were analysed with regard to the presence of hemodynamically significant coronary lesions. Specifically, each abnormal myocardial segment was assigned to the pertinent vascular territory by spatial co-

registration according to patients' individual coronary anatomy. A matched hybrid imaging finding was defined as an inducible perfusion defect in a territory subtended by an obstructive stenosis (>50%) on CTA. All other combinations of pathologic findings were classified as unmatched.

Statistical analysis

Categorical variables are presented as numbers (percentage), continuous variables as mean±SD or median [25-75 percentile] depending on their distribution.
Differences in continuous variables between two groups were tested using Student's t test or Mann-Whitney test. Comparisons among groups were performed using ANOVA and Kruskall-Wallis test. Bonferroni test or Mann-Whitney test using Bonferroni correction for P-value were used for post-hoc comparisons. Pearson's chi-squared test was used to compare categorical data.
Linear regression was used to estimate the effect of clinical, biohumoral variables as well as imaging results on hs-cTnT and NT-proBNP levels. All multivariate models were developed considering variables with a P value <0.1 at univariate analysis and then using backward and forward stepwise selections to build up the final models. The logarithmic transformation of continuous variables was used in linear regression analysis.
All analyses were performed using StataCorp. 2007. A 2-sided value of P<0.05 was considered statistically significant.

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